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Leaf water and stomatal movement in Gossypium and a method of direct visual observation of stomata in situ

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In a previous paper* it is shown that the rates of transpiration in cut shoots of the ocotillo, *Fouquieria splendens*, recorded by simultaneous volumetric readings and weighings, are not parallel, but that the loss of water from the plant during the day is in excess of that taken up by the cut end of the shoot from the porometer. This result is in general harmony with the findings of Eberdt† with rooted plants of *Helianthus annuus*. It was not, however, found to be true in my study of the ocotillo that the loss of water takes place at a constant ratio during the hours of daylight, since the whole relation between the income and outgo may be reversed within a short space of time even during daylight by an apparently slight modification of the environmental conditions. This ready susceptibility of the plant lent color to the idea that the differences indicated by volumetric and gravimetric readings are measures of differences in the leaf moisture content, to be more briefly referred to as leaf water in the present paper. Determinations of the amounts of water held within the leaf at various times of the day, these being calculated in percentages of dry weight, showed that such expected differences do occur; but the evidence was incomplete in that the amounts of leaf water were not calculated to a constant, since it must be assumed that

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[The BULLETIN for December 1912 (**39**: 567–631. *pl. 40–45*) was issued Jan. 2.]

* The relation of transpiration and stomatal movements to the water-content of the leaves in *Fouquieria splendens*. Plant World **15**: 1–14. Ja 1912.

† See Burgerstein, A. Die Transpiration der Pflanzen 17. Jena, 1904.

the dry weight of the leaf increased during the day. The conclusions drawn rested more especially on the assumption that the leaf water at the close of day is not in material excess of that at the beginning, and upon the fact that during the afternoon there is a sharp rise in the amount of leaf water in spite of a constant, if not a still increasing, dry weight. The hope that during the summer of 1911 the more convincing data based upon constant leaf area could be obtained was not realized, and the evidence therefore still remains incomplete.

In September 1910 a similar attack was made upon the cotton plant at Auburn, Alabama, in which analogous conditions with respect to leaf water were found. During the present year the question has been further studied, the material being obtained from a pure strain of Dixie cotton, the seeds of which were supplied me by the Bureau of Plant Industry.

Plants were grown at Auburn and, under irrigation, at Tucson, Arizona, in the experimental grounds of the Desert Botanical Laboratory of the Carnegie Institution of Washington. My thanks are due to Dr. D. T. MacDougal for his courteous cooperation. The present paper reports upon a portion of the data obtained bearing upon the variation in the amount of leaf water and upon the behavior of the stomata.

METHODS

DRY WEIGHT AND LEAF WATER. The first two series of leaf samples, the figures for which are given in TABLE I, consisted of leaf material placed in tared, cork-stoppered bottles, weighed, oven-dried thoroughly and reweighed. The samples were taken at two hour intervals. The actual work was done by Mr. C. S. Ridgway, to whom my thanks are extended. The samples on which TABLE III is based were taken at assumed critical times, and treated in the same manner by myself.

The data in the remaining tables were obtained by cutting circles of the lamina with a sharp cork borer, placing them forthwith in tared vials and weighing before and after thorough drying. The area of these circles, of which between fifty and sixty were taken for each sample, was calculated, and the exact number of circles taken determined a second time after drying. All data were calculated to 100 square centimeters of leaf blade. In this way the changes in dry weight and in leaf water for each period were determined.

STOMATA. The measurements given in TABLE IX are average micrometer measurements of a large number of stomata for each period, made on material fixed in absolute alcohol.* Those in TABLE X are measurements of living stomata made in the field by the following method, first hit upon by me during my work at the botanical laboratory of the Carnegie Institution of Washington, at Carmel, California, during the summer of 1911.

A microscope, provided with a condenser and a 4 mm. objective with long working distance, was provided with a cooling cell. This was extemporized by wiring a Soyka flask beneath the substage apparatus. For working in the field, a heavy camera tripod served as a stand capable of ready adjustment in height and position. The illumination consisted of direct sunlight, or when that was not available,. a strong artificial light. I used a small miner's acetylene lamp, on the whole fairly satisfactory. A small arc light probably would be better, and indeed necessary when the leaves are very thick.

By means of such strong illumination, properly centered and controlled with the iris diaphragm, it is possible to measure accurately living stomata of leaves with a thickness of 5 mm. without at all injuring them. The method is adapted to the observation of the stomata on a great variety of leaves, including many which are densely covered with trichomes (e. g., *Parthenium argentatum* Gray; *Chenopodium* sp.), which being out of focus do not interfere with the vision under strong illumination. The stomata at the bottom of deep pits, such as are found in the Cactaceae, also can be seen, but so far this has involved the removal of a thick slice of the underlying tissues.

The method presents the further advantage, one of great importance in field work, of permitting the repeated observation of the living stomata, or indeed of a single one, during a sustained period, without injury to the leaf or its removal from the plant. This method has, of course, its limitations in view of the size and shape of the microscope,* but these limitations are not as strait as one might suppose. For the direct visual observation of the living stomata *in situ*, for any reasonable desired period, the method above outlined has a unique value for certain purposes. It will be found, I venture to believe, to be invaluable in supplementing and checking the results obtained by such methods as that of alcoholic fixation (Lloyd, *l. c.*), of the hygroscope (of whatever type), of the porometer,† and of the stomatograph.‡

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*Lloyd, F. E. The physiology of stomata. Carnegie Inst. Wash Publ. 82. 1908.

In using the method one must have a care for his eyes. The adjustment of the illumination must be done with the protection of a color screen, and for this purpose a leaf interposed in front of the objective has served well enough. Under field conditions, especially when one is working for a considerable length of time, dark eyeglasses are very desirable, more particularly if the surroundings are highly illuminated. Small circular discs of dark-tinted glass in various shades to be placed above the ocular have been found advantageous, more especially when the leaf is rather thin, say as thin as the leaf of the cotton.

In the actual observation of the stomata the leaf is placed directly on the stage without any accessories in the form of glass slides or covers. It is held in position by gentle pressure upon it of the index and middle fingers, which, also, embrace the end of the objective. In the eyepiece a micrometer is placed. The absence of a glass slide, which is in any event unnecessary, brings the stomata nearer the focus of the condenser and so improves the definition. As one is dealing with a brilliant area of light, it is quite necessary to have it central, since oblique illumination causes bright reflections from the surface of the stomatal outer vestibule, which may mislead the observer.

LEAF WATER IN PERCENTAGE OF DRY WEIGHT

If we consult TABLE I, which contains the percentages of leaf water calculated to dry weight for two parallel series, treated as nearly alike as possible, it will be seen that when so expressed it varies between the extremes of 318 and 220 per cent. The greatest difference between the two figures for any hour is 26, this greatest discrepancy being found at the 8 and at the 14 hour on September 17. If we assume an error of 13 units and apply a corresponding correction by addition to the smallest and by subtraction from the largest percentage, a difference of 72 units is still afforded. If the correction is similarly applied to the percentages in either series, the difference may be reduced to 46 units, as between the 8 and 14 hour figures. It will appear from these figures that the error of observation is not great, and is probably within five per cent., a conclusion well sustained by the general correspondence of the figures for the two series. It is, further, probably entirely true that the differences are not due to a material error of measurement, but are actual differences in the leaf water of different parcels of material. Since these were not taken by myself, I have compared the differences with those displayed by my data for the ocotillo, upon which I based my conclusions embodied in my paper above referred to,* and I find that discrepancies in similar amounts are to be found in them. The same is true of the data which appear in the present account beyond. They may be due to individual differences, or to differences in groups of plants under various external conditions, or in the positions of the leaves taken. The general correspondence between the two series (FIG. 1) is, however, sufficiently close to warrant drawing the conclusion that during the night there is a rise in the leaf water content (which probably had been preceded by a greater increase previous to the 16 hour) and a decided decrease during the day till 2 P. M. The sharp rise and equally sharp fall indicated between the 18 and 22 hours is puzzling, since if we refer it to change in dry weight, the additional difficulty is introduced of accounting for marked changes in the same. Aside from this peculiarity of behavior the general rise in water content may be referred to the reduction of dry weight, but this involves the assumption that the dry weight was reduced much more rapidly in the period preceding the 18 hour (say between the 14 and 18 hour) than subsequently. A somewhat similarly sharp

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* An instrument of special design, now in course of construction, will obviate many inconveniences.

† Darwin, Francis, and Pertz, D. F. M. On a new method of estimating the aperture of stomata. Proc. Roy. Soc. B. 84: 136–154. 1911.

‡ Balls, W. L. The "Stomatograph." Nature 87: 180. 10 Au 1911.

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* The relation of transpiration and stomatal movements to the water-content of the leaves in *Fouquieria splendens*. Plant World 15: 1–14. Ja 1912.

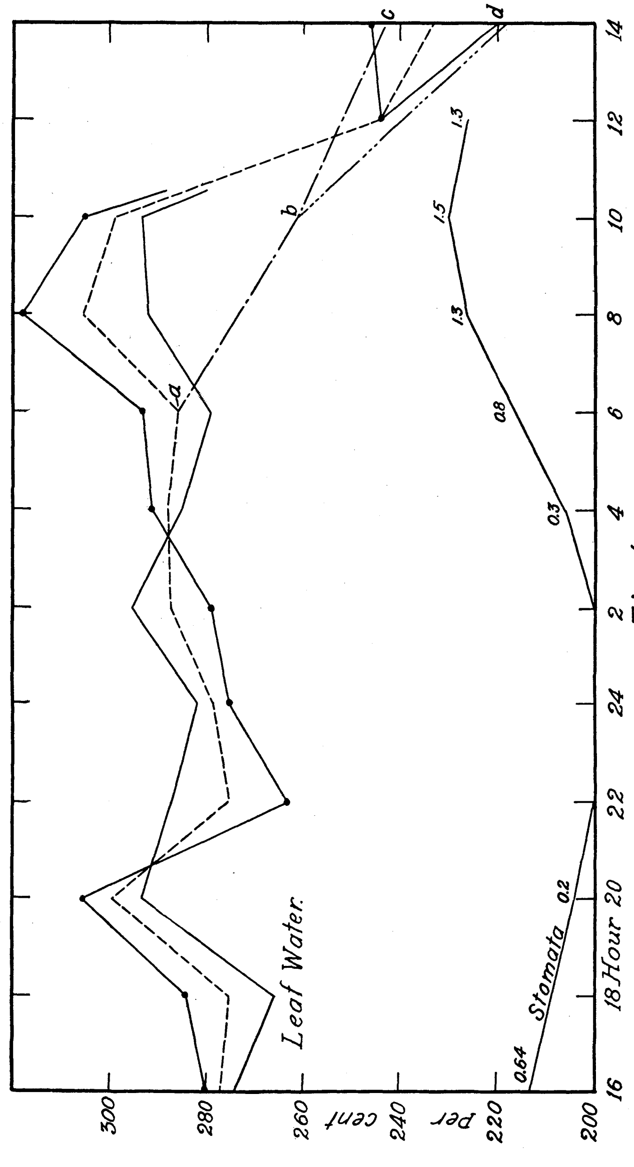

Fig. 1.

Gossypium herbaceum. Graphs for leaf water for two series and for their averages; and for average transverse stomatal dimensions.

rise in water content is indicated after the 6 hour, September 17, continuing till 8. The tables following show such behavior in only one instance (series 12), but it must be understood that the data therein contained were based on material collected on bright days, while those now being considered were taken on a morning (of September 17) during the first few hours of which a general haze prevailed. The single exception, offered by the old leaves collected on October 13, 1911 (series 12, TABLE VIII), differs quantitatively, there being here a difference of 15 units (251 to 266 per cent.) as compared with 19 units (284 to 303 per cent.) in the case before us of September 17.

The value of the comparison is slightly compromised by the fact that the data of TABLE VIII indicate a decrease of dry weight, in an amount that may, however, fall within the limits of error. If no change in dry weight is assumed, the difference in water content in percentage of dry weight would be somewhat reduced.

In view of the further fact that the data for both TABLES V and VIII were obtained after an increase in soil moisture, it seems entirely possible that under certain conditions the first two hours of daylight may be accompanied by an increase in leaf water. This involves as a corollary that the leaf, at the hour of sunrise, may not be completely plethoric of water.

In order to evaluate with some degree of exactness the meaning of the graph embodying the data of TABLE I, I have assumed that on September 17 the dry weight of the leaves increased in the amounts of 9 per cent. on the original weight in the first four hours (6–10) and 17 per cent. in the whole period of eight hours, namely 6–14. These amounts are taken from TABLE VII, series 8, and represent fairly closely the average performance for young leaves. The weighings for the 6 hour are taken as a basis for the calculation, and the leaf water content is assumed to remain constant, the dry weight only increasing. By comparing the ratios thus obtained with those actually observed, as displayed in TABLE II, we see that the calculated value of the ratio at the 10 hour is less than the observed, and at the 14 hour greater (graph *abc*, FIG. 1), the latter indicating a net loss of leaf water. If however, an increase of 30 per cent. on the original dry weight be assumed, an assumption apparently justified in view of the data in other tables, a net loss of water would not be discoverable (graph *abd*, FIG. 1). It is evident, however, from the directions of the graphs that a negative fluctuation was in progress from the 10 to the 12 hour, followed by increase afterwards. It is also evident that a positive fluctuation begins after the 14 hour, if we suppose the datum for the 16 hour, September 16, to apply approximately to the same hour on September 17.

Although the above evidence is quantitatively somewhat vague, this result is probably due to the application of assumed dry weights based upon data obtained in the following year. Had such data been obtained at the time, their employment would lead to a surer inference. This appears from similar manipulations of the determinations contained in TABLE III of leaf water and dry weight for unknown leaf areas.

In the first three columns are given the known data; in the fifth the average dry weights from TABLE V, which were obtained two days later, these being relative to 100 square centimeters of leaf and assumed to be applicable to August 24. Since the two days (August 24 and 26 at Tucson, Ariz.) were practically identical meteorologically, this assumption is probably entirely justified. By dividing the assumed dry weight into the observed dry weight for each hour the approximate area of the leaf sample taken at that hour is obtained. This affords one term of a ratio (column 4), the other of which is the observed leaf water (column 3) by which the leaf water per 100 square centimeters is derived. This, for each of the three hours of observation, is found in column 7 and is plotted in graph 1, FIG. 2.

Just above this is graph 1*a*, the coordinate points for which were determined as follows. (See TABLE IV.) The same ratios of increase of dry weight were assumed as above. One of the observed dry weight readings in TABLE III was then taken as a standard, and the dry weights for the other two hours were determined by means of these ratios. By assuming the leaf water to be constant the inverse ratios, expressing leaf water as percentage of dry weight, were obtained (column 2, TABLE IV). By comparing these with the ratios (column 4) of the data recorded for each hour (columns 2 and 4) differences are obtained by subtraction which determine the position of the graph of observed leaf water

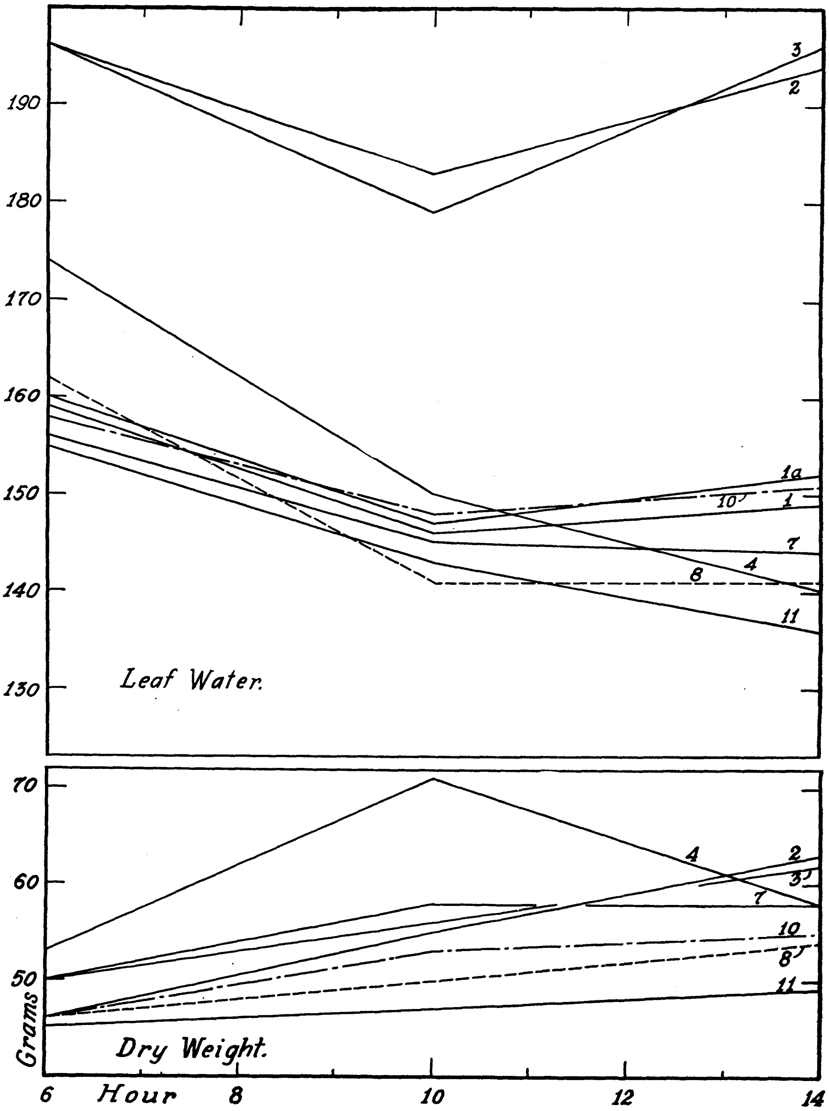

Fig. 2.

Gossypium herbaceum. Graphs for leaf water and dry weights of leaves for the series indicated by numbers corresponding to their numbers in the tables. The ordinates in this and FIG. 3 are the same for the upper and lower portions but have been shortened between 70 and 130 grams. Young leaves.

relative to the graph of constant water when the latter is assumed to be horizontal, as it would be if plotted relative to constant leaf area (column 6). By making the value of the 6.20 hour figure say 160, we obtain results which differ little from those in column 6, TABLE III, in calculating which a variable due to a somewhat inconstant relation between leaf weight and area was admitted.

It is apparent that a much larger increase in dry weight for the first four hours of the day than that assumed, together with far different march of events during the whole period, must have obtained in order to obviate the conclusion that on August 24 there was a net reduction in the amount of leaf water followed by a material recovery.

In addition to graphs 1 and 1*a* are to be found in FIG. 2 the graphs for the young leaves in the remaining tables. The general similarity of the lot further impels the conclusion that graphs 1 and 1*a* are not wide of the truth.

I have taken the above somewhat indirect method of drawing the conclusions desired with reference to the observations recorded in TABLE III, for the purpose of developing a comparison of the observations for the two days, 24 hours apart, at Tucson. We have already examined those of August 24, presented in TABLE III. Those for August 26 are to be found in TABLE V, in which the weights are relative to 100 sq. cm. of leaf blade. Although there was a marked fluctuation in the leaf water on August 26, the loss of water between the 6.20 and 10.20 hours being as great as that on August 24, it is to be noted that the recovery after the 10.20 hour was more rapid on August 26 (graphs 2, 3), and that the absolute amount of leaf water was considerably greater, viz. about 25 per cent., on this day than on August 24. Since the leaves from which the samples were taken were of uniform texture and at similar stages of development, this difference cannot be attributed to variation in these. The difference is due quite probably to the circumstance that on August 25 the ground in which the plants were growing was irrigated. This, if the proper explanation, carries with it the suggestion that it would be well worth while to determine with what precision the soil water conditions may be registered in the condition of the leaves. It is quite possible that the needs of the plant for a water supply to its roots could be determined by following the changes in leaf water from day to day, and so anticipate a condition of necessity which might curtail growth for a period. Obviously such a procedure would be of practical use only where water is under control.

We now turn to consider the tables which present data relative to a constant leaf area, namely 100 square centimeters.

For the purpose of comparison in what follows, the data embodied in TABLES V–VIII inclusive have been segregated in two sets of graphs, those (in FIG. 2) for young or full-grown but not old and indurated leaves, and those (FIG. 3) for old leaves, rather the worse for wear. They were indurated, torn and more or less discolored, but a consistent effort was made to collect the material from leaves with a healthy appearance. The three series (1 to 3 incl.) for Tucson, Arizona, are included in FIG. 2, since the leaves, while mature, were not at all indurated and, indeed, had perhaps not arrived at full growth, though they were well developed.

Of the seven series of samples from young leaves one only, viz. no. 4, appears to indicate an aberrant behavior, especially as to increase in dry weight, but evidently so as to the loss of water also. If we disregard this case, for which no explanation is offered, the remainder show a general accord. Of the six remaining cases, two (2 and 3), those for Arizona, show an increase considerably in excess of those for Alabama. The ratio of increase for Arizona is 100 to 120 for the first four hours and 100 to 136 for the whole period of eight hours, beginning at 6.20 for the first series; the ratios for the second series are 100 to 112 and 100 to 124. In Alabama the greatest gain for a like period was in the ratio of 100 to 121, while the lowest was from 100 to 109.

Stated in terms of increase of photosynthates, we have for the Arizona plants an accumulation at the rate of 2.25 grams per hour per square meter for the first four hours, and 2.00 grams for the second four hours, for no. 2, TABLE V; the corresponding figures for no. 3 are 1.50 and 1.50. For the young leaves of Alabama plants, excluding series 7, TABLE VI, the range of increase (when there was any increase at all) is from 0.5 to 1.6 grams. Sachs' determinations for the sunflower were 1.88 and 1.7, and for the cucumber 1.5 gr. Brown and Escombe's datum for the sunflower is, however, 0.714 gr. Although my own data were

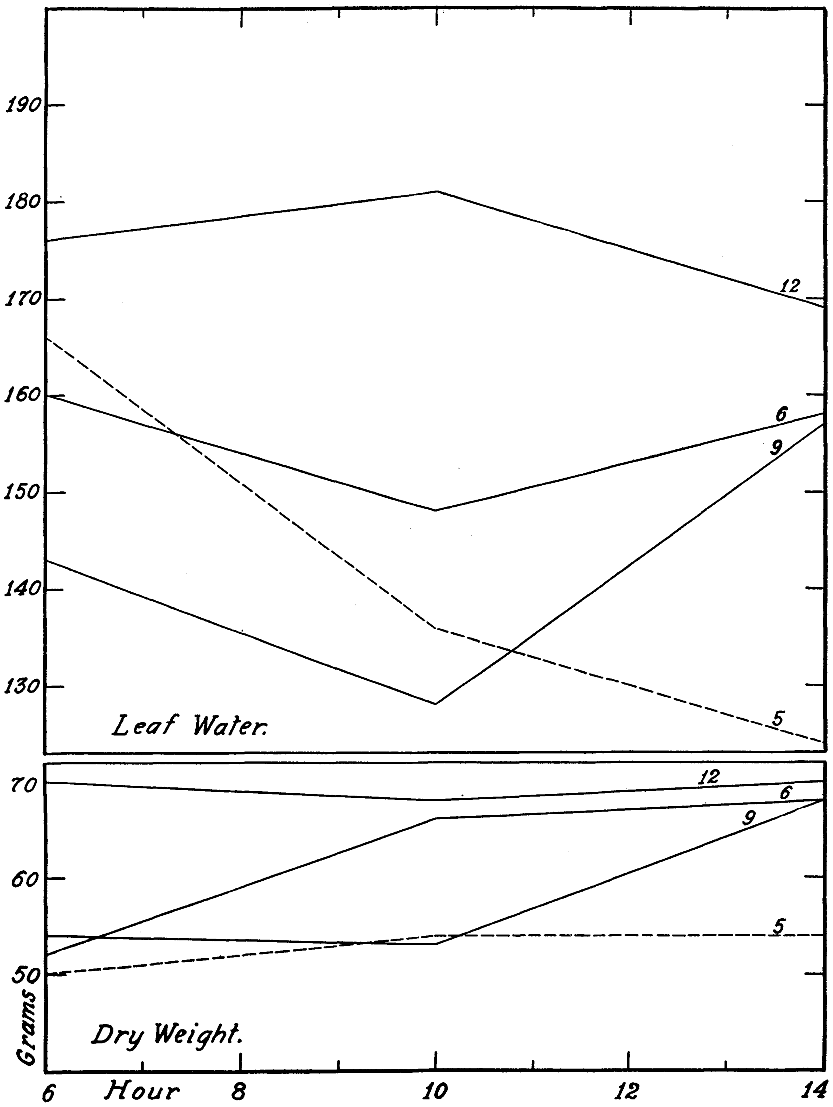

Fig. 3.

Gossypium herbaceum. Graphs for leaf water and dry weights of leaves for the series indicated by numbers corresponding to their numbers in the tables. Old leaves.

not obtained with a view primarily to obtaining light on the accumulation of carbohydrates, the general correspondence of the Alabama figures with those quoted may be noted. The greater efficiency of the Arizona plants possibly may be due to the fuller development of the leaves. This would be indicated by the performance of the old leaves on October 1 and 8 (series 6 and 9), which showed an increase of 30 and 26 per cent., but more surely, if it were not for the general irregularity of their behavior and the entire failure to get an increase at all in series 12 and only a very slight one in series 5. On the other hand the light conditions in Arizona are much more favorable in view of the frequent cloudiness in Alabama. The days on which the Alabama material was collected were partially cloudy, while that from Arizona was taken on continuously clear days.

The old leaves betray a much more erratic behavior and the data lead us to no safe conclusion. In the two cases, TABLE VI, series 5; TABLE VII, series 9, however, in which there was a marked increase in dry weight, there was also a marked recovery of leaf water after the 10 hour. By contrast, however, the leaves that contained the greatest quantity of water, series 12 of October 13, were the least efficient photosynthetically, a circumstance which throws doubt on the inference that the failure of the leaves of series 5, taken from all parts of the plants, to add to their dry weight after the 10 hour, was due to a marked loss of water during the corresponding period.

STOMATAL MOVEMENT

A glance at FIG. 2 will show that after the 10 hour has been reached there is a distinct tendency to recover the leaf water lost during the earlier hours. It is important to inquire whether this tendency, which begins to make itself evident during the part of the day when transpiration rates may be assumed to be the highest, is in correlation with the behavior of the stomata.

Balls,* in Egypt, found by means of his newly devised stomatograph that the stomata of the cotton slowly close as the hottest part of the day approaches, completing the closure as the sun passes behind neighboring trees at 13.40 hr., remaining closed all the succeeding night. They open slowly after sunrise, but more rapidly after the sun strikes the leaf at about 7, attaining the maximum opening at 9, but thereafter closing steadily in spite of brilliant illumination. So far as I am aware this is the only record of the behavior of the stomata in cotton.

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* Balls, W. L. The "Stomatograph." Nature 87: 180. 10 Au 1911.

The measurements given in TABLE X, made microscopically and at the same time by two observers, Mr. C. S. Ridgway and myself, in the manner earlier described, correspond in a rather striking way with Balls' conclusions, just indicated. Each formula represents the minimum and maximum width of the stomatal pores on the upper and under sides of the same leaf. When a very few stomata displayed extreme dimensions, these are enclosed in parentheses. It may be regarded that these would on account of their small numbers affect but little the general diffusive capacity of the stomata taken as a whole.

It is evident that during the night and until 6.20 there was no measurable opening movement. This must not be taken to mean that all the stomata were entirely closed. TABLE IX gives stomatal dimensions from preparations made by the alcohol method, in which it is evident that some opening in a few stomata may be observed at any time during the night. The initial opening on September 30, 1911, occurred about 6.30, from which hour on a progressive opening movement was followed, the stomata of the lower faces opening somewhat in advance of those of the upper, though some exceptions to this appear, as at 7.50 and 8; while Livingston and Estabrook* found, in several kinds of stomata, that the upper close and open more rapidly or close more completely than the lower. The maximum average pore width was attained during the period between 7.45 and 9, though the widest openings were seen at 8.20. Because of the extreme sizes of opening then noted, leaves of similar size and exposure were examined to verify the observation, but they were no more seen during the morning, except in one instance an hour later. The closing phase was at once entered upon, but it is evident from the figures that this movement was less uniform in all the leaves taken together than the opening. This may be readily understood when it is appreciated that the stomata of cotton are quite sensitive. I observed, both in Alabama and in Arizona, that at the same hour a leaf in the shade and one in the sun were physiologically antipodal in this regard. Thus, at 8.30, August 24, at Tucson, Ariz., the stomata of a leaf in the shade were closed both above and below, while those fully exposed to sunlight on another leaf were 0–10μ wide above, and 2–4 μ below. Similar conditions are displayed by the leaves observed at the 10 hour, TABLE X. At the same hour it was noted that the stomata near the apex of a leaf were closed while those near the base were open, a condition readily understandable if wilting is progressive from the apex of the leaf downward.

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* Livingston, B. E., and Estabrook, A. H. Observations on the degree of stomatal movement in certain plants. Bull. Torr. Club **39**: 15-22. 12 F 1912.

As between young and old leaves, the latter being still physiologically active, it is not clear that there was a more pronounced tendency in one kind than in the other either toward opening or closing, nor that the leaves near the base of the plant were more favorably placed than those three feet above, at or near the top. Indeed, that the upper leaves close their stomata less early than those near the base seems indicated, and this is in harmony with Pringsheim's findings,* who believes that the apical parts of the plant withdraw water from the older, the result of more rapid transpiration from the younger organs.

The data of the table which we have been considering must be regarded, however, rather as an indication of the usefulness of the method of microscopical observation in the field after the manner already described. The measurements were taken rather at random, and as the plants were nearing the end of their career, the larger leaves being somewhat *passées*, while the young were from "second growth" shoots, at the tops of the plants. Their general indications, however, I believe, may be relied upon, namely that the maximum opening of the stomata is reached at about 8.30 in the morning. It has been the invariable experience of three observers during the season of 1911 at Auburn, and of one of them at Tucson, that wilting of the leaves becomes first evident at about this hour or somewhat later each day, when sunlight conditions prevail. The reduction of leaf water leading to this condition* has been demonstrated quantitatively above, the minimum amount being found, in these determinations, at the 10 hour or somewhat later. It becomes evident that the opening of the stomata is accompanied by a net loss of water by the leaf, more being given off by transpiration than can be obtained to replace it, as I have shown to occur by comparative volumetric and gravimetric determinations in *Fouquieria splendens*.† The results of similar experiments with cotton may be said, in anticipation of their publication, to harmonize fully with those on *Fouquieria*.

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* Pringsheim, E. Wasserbewegung und Turgorregulation in welkenden Pflanzen. Jahrb. Wiss. Bot. 43: 89–144. 1906.

Concerning the relative behavior of young and old leaves it may be further pointed out, that so far as the evidence goes in the present paper there is little against either of the alternate views that this loss of leaf water is better resisted by the young or by the old. The numbers of stomata per unit area in the young leaves are considerably greater. An important inquiry at this point is in regard to the relative extent of the internal evaporating surfaces,‡ but though we do not find the answer, we may be sure that it is sufficient in young leaves to lead to wilting, and that the stomata do not head off the net loss during the period of advancing temperature, insolation, and evaporating power of the air.

It is quite possible that determinations of the amount of leaf water at smaller intervals of time would lead to the finding that the loss is more sudden, after it once begins, in old leaves than in young ones, and that this may be connected with the relative diffusive capacity of the stomata in consecutive intervals of time. I have arrived at this suggestion by a comparison of the dimensions of the slightly open pores of young and old stomata, and by calculating their relative diffusive capacities.§ It develops that in old stomata the edges of the pore are more stiffly reinforced, and that in opening they separate from each other throughout nearly their whole length at once, making a long narrow opening. Thus I find that the pore lengthens from 12 to 13.5 μ in one case, and from 9 to 10.5 μ in another, while the width reaches 1.8 and 2 μ respectively, these transverse measurements being applicable throughout the major portion of the length of the pore. Such stomata probably attain a relatively high diffusive capacity more quickly than young ones, in which pores, because of the flexibility of their edges, they attain their full length gradually. In the only series of preparations I have at hand the shortest pore I find is 4.5 μ long and 1 μ wide. Its index of relative diffusive capacity is 2.12. Assuming that in further opening its dimensions advance to 8.4 by 1 μ (those of a similar stoma in the same preparation) and then to 9 by 1.5 μ, the indices would be, for these dimensions, 2.9 and 3.67, whereas an older stoma would have measurements such as 14 by 0.8 μ, or 12 by 1.5 μ, with the indices 3.35 and 4.24, respectively. That is, for the same width of pore, the maximum length is reached much more rapidly and a relatively high diffusive capacity more quickly in older than in younger stomata. If relations such as these obtain, as the evidence before us appears to indicate, we may well think that they may affect the rate of incipient drying,* either with or without appreciable wilting. Differences of this kind may help to account for the results obtained by Bergen,† who found that the old leaves of sclerophyls transpire more than young leaves which have just attained their maximum area, though he further observed that the same relation holds for cuticular transpiration as well. In this regard, Pringsheim's work, already referred to, may not be overlooked. It is to my mind doubtful, in view of the results on loss of leaf water presented in this paper, that the differences observed by Bergen‡ as between sun and shade leaves depend upon the water carrying capacity of the vascular tissues.

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* Livingston, B. E. Relation of the daily march of transpiration to variations in the water content of foliage leaves. Bot. Gaz. 53: 309–330. 1912.

† Lloyd, F. E. The relation of transpiration and stomatal movements to the water content of the leaves in *Fouquieria splendens*. Plant World 15: 1–14. Ja 1912.

‡ Bergen, J. Y. Relative transpiration of old and new leaves of the *Myrtus* type. Bot. Gaz. 38: 446–451. D 1904.

§ Livingston, B. E., and Estabrook, A. H. Observations on the degree of stomatal movement in certain plants. Bull. Torr. Club 39: 15–22. 12 F 1912.

Turning to the data of TABLES I and IX, as represented in FIG. 1, it becomes apparent that the sharp rise in leaf water between the 6 and 8 hours neither harmonizes with the results given in the remaining tables, nor does the curve of stomatal openings for the hours of observation bring out the relations between these and loss of leaf water in sufficient detail. That there should have been increase in leaf water in the amount of about 7 per cent. during the period (6 to 8) when the stomata were opening from 0.7 to 1.4 μ, must have been due to meteorological conditions, though it is still not clear why the maximum leaf water had not been attained by the 6 hour, unless it should be shown that such a condition does not always intervene even in the early morning hours before sunrise.

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* Livingston, B. E. *l. c.*

† Bergen, J. Y. Relative transpiration of old and new leaves of the *Myrtus* type. Bot. Gaz. **38**: 446–451. D 1904.

‡ Bergen, J. Y. Transpiration of sun leaves and shade leaves of *Olea europaea* and other broad leaved evergreens. Bot. Gaz. **38**: 285–296. O 1904.

The flatness of the curve of stomatal opening after the 8 hour, it is likely, does not properly represent the facts, since from the evidence in TABLE X there was probably a considerable opening and closing between 8 and 10, with the maximum diffusive capacity reached at about 9. If, aside from this, the curve of stomatal opening is true, it would seem to indicate that the loss of leaf water from 300 to 240 per cent. took place without a sufficient amount of wilting to involve closure of the stomata, and that the leaf therefore had not passed beyond the condition of drying incipient to wilting. As no observations were recorded to test the truth of this conclusion and no measurements of the meteorological conditions beyond the note that there was a general haziness until the 10 hour were made, we perhaps may be permitted to draw the lesson, that in such studies it is of utmost importance that the integral effect of such conditions should be known before any adequate interpretation of the water relations of the plant may be had. At present the most efficient method to this end is that of measuring evaporation with the blackened porous cup.* In a future paper it is proposed to display the results which have been obtained in field studies of cotton in the light of such integration.

Reference has been made above to the daily wilting of the leaves, observable at first at about the 9 hour. The stomatal measurements would seem to indicate that after this hour there is a rather steady closure. It therefore would be of importance to determine at what hour the maximum transpiration rate occurs when these conditions prevail. In anticipation of such determinations, now possible by the use of the method I have described above, and with reference also to F. Darwin's* verification in cooperation with Pertz, by means of the porometer, of his earlier work on the temporary opening of stomata during wilting, I measured by weighing the rates of transpiration of a piece of a cotton plant during wilting. After the cutting off, the cut was greased and the piece was suspended on a balance, the loss of weight being determined at intervals of several minutes, beginning at 7.22 when the stomata were open but had not reached their maximum. The leaves were turgid. The figures obtained (TABLE XI) show that between 8.02 and 8.07, following a steady fall in the rate, there was a temporary rise, and while this was very small when compared with Darwin's data, it may be a phenomenon comparable with that observed by him. However, that the stomata open after wilting sets in still appears doubtful to me. I have watched microscopically the stomata of the cotton, in addition to those of several other species, during the wilting period, beginning with the partly as well as fully opened condition, and I have never observed any such movement. There is at first no change. As wilting sets in, closure commences and proceeds steadily till complete, and I have observed no subsequent opening. Obviously further investigation is required to throw light on these differences.

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* Livingston, B. E. A radioatmometer for comparing light intensities. Plant World 14: 96–99. Ap 1911; Light intensity and transpiration. Bot. Gaz. 51: 417–438. D 1911.

CONCLUSIONS

In the foregoing paper a method for the direct observation and measurement of stomata *in situ* is described. The method is adapted to field work by night and by day.

Leaf water, stated in percentage of dry weight, was found to vary in the cotton plant under usual conditions between 318 and 220 per cent. On the day of observation the minimum leaf water content was reached at the 14 hour or thereabouts. This reduction represents a net loss as shown by the determinations made relative to unit area and, therefore, with quantitative regard to dry weight.

The amount of loss of leaf water when thus determined is from 7 to 15 per cent. on the initial amount at sunrise, under the conditions prevailing when the observations were made. Among these conditions it may be mentioned incidentally that the soil was well drained and rich in moisture at the time. Severer circumstances would no doubt effect a still greater loss. The observed loss, however, may be taken as indicative of a usual phenomenon, the reality of which is made evident in an observed daily wilting of the leaves beginning at about the 9 hour, detectable not alone by change in position (since this may occur as a phototropic response) but by flaccidity. The case may be otherwise stated by saying that under usual day conditions, with sunshine, the roots are unable to supply loss of water from the leaves. Balls'* view that the water supply is the limiting factor of growth, and his observation that no growth takes place under the Egyptian sun appear to be quite applicable to Alabama. With regard to the amount of growth in Alabama, preliminary measurements, prompted by the results obtained from the determinations of leaf water, indicate that even under the presumably more favorable humidity conditions obtaining here growth does not take place for the major portion of the day, since during the latter part of the growing season an actual daily shrinkage in stem and leaf length has been observed. I can hardly concur, however, with Balls in his view that because growth does not take place in sunshine this is to be interpreted as unfavorable. Comparative measurements on the same variety of cotton obtained in Arizona betray a no more unfavorable reduction of leaf water than in Alabama, when there is sufficient water in the soil. There is evidence that variations in soil moisture are registered in both the absolute leaf water content and in the rate of recovery after the minimum quantum for the day has been reached, while the loss during the first part of the day appears to be less affected. It would seem that the real test is the growth integer for the season, and it is not evident that, with irrigation, the conditions in the semiarid Arizona desert at all events are unfavorable from this point of view. A hot sunshiny day after all may be good for cotton, but this good may not be apparent in growth at the time. This is indicated by the amount of photosynthates formed (measured with small error due to well understood causes) by the increase in dry weight. Two series in Arizona gave increases of 24 and 32 per cent. for 8 hours. In Alabama, with the exception of one series of old leaves in which there was no apparent change in weight, the increases ranged from 6 to 25 per cent., the latter being one instance out of a total of seven series. Whatever may be said of increase in dimensions, therefore, it remains the fact, that in spite of the hot unmodified Arizona sun shining throughout continuously cloudless days in August, more energy in the form of carbohydrates was made available than in the similar periods in Alabama. It is proper, however, to recall that the leaves studied in Arizona were of fuller development, while those in Alabama were either rather young (10–15 cm. transverse measurement) or overmature.

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* Darwin, F., and Pertz, D. F. M. On a new method of estimating the aperture of stomata. Proc. Roy. Soc. B. **84**: 136–154. 1911.

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* Balls, W. Lawrence. The physiology of the cotton plant. Cairo Sci. Jour. 4: 1–9. Jl 1910; Cotton investigations in 1909 and 1910. Cairo Sci. Jour. 5: 221–234. S 1911.

It is obvious that it will be of great interest to make careful measurements of growth, as indeed of other functions, for comparison with those of Balls in Egypt.

The stomata are practically closed at night, but nevertheless show a tendency to open during the early morning hours.* The more obvious daily opening begins at about 6.30, in Alabama in September, and the maximum is reached at about 8.30 or 9, after which closure progresses until 11 or somewhat later. A concomitant and appreciable wilting takes place, correlated with the reduction of leaf water. During wilting there appears to be no "temporary opening" of the stomata, although I have observed a measurable but not very marked rise in the rate of transpiration about a half hour after wilting starts in, followed by a sudden reduction of rate.

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*Lloyd, F. E. The physiology of stomata. Carnegie Inst. Wash. Publ. **82.** 1908; Darwin, Francis, and Pertz, D. F. M. On a new method of estimating the aperture of stomata. Proc. Roy. Soc. B. **84**: 136–154. 1911.

TABLE I

Gossypium herbaceum. Auburn, Ala., Sept. 16–17, 1910. Leaf water in percentage of dry weight.

Hour	Series I	Series II	Average
16	274	280	277
18	266	284	275
20	293	306	299
22	287	263	275
24	282	275	278
2	295	279	287
4	285	291	288
6	279	293	286
8	292	318	303
10	293	305	299
12	244	244	244
14	220	246	233

TABLE II

Observed leaf water expressed in percentage of dry weight and the percentages obtained by assuming a 9 per cent. increase in dry weight for the first four hours and 17 per cent. and 30 per cent. for the second four hours.

Observed				Calculated		
Hour	Dry wt.	Water	Per cent. water	Dry wt	Water	Per cent.
6	4.15	11.79	284			
10	3.70	11.07	299	4.52*	11.79	261
14	5.53	12.87	233	4.86†	11.79	243
	5.53	12.87	233	5.40‡	11.79	218

TABLE III

Gossypium herbaceum. Series 1, Tucson, Ariz., Aug. 24, 1911. Amount of leaf water in percentage of dry weight and the same calculated to 100 sq. cm., the average dry weights in TABLE V, being assumed as applicable to the material on which this table is based.

Hour	Observed weights		Area	Weight per 100 sq. cm.	
	Dry	Water		Dry (assumed)	Water
6.20	10.55	34.36	2153	0.49	1.59
10.00	10.84	28.02	1918	0.565	1.465
13.30	12.12	28.78	1933	0.627	1.493

* 4.15 plus 9 per cent.

† 4.15 plus 17 per cent.

‡ 4.15 plus 30 per cent.

TABLE IV

Leaf water relative to constant area computed from the observed weights in TABLE III. The average rate of increase in dry weight recorded in TABLE V is assumed.

Hour	Ratios of water (= C) to dry weight		Observed ratios water to dry weight		Water to dry weight, area = C	Leaf water per 100 sq. cm.
6.20	326	100	326	100	100	1.60
10.00	285	87.5	259	79.5	92	1.47
13.30	254	77.7	238	73.0	95	1.52

TABLE V

Gossypium herbaceum. Tucson, Ariz., Aug. 26, 1911. Weight of water and dry weight in grams per 100 sq. cm. of leaf at the hours indicated. Two series Irrigated Aug. 25.

Series	6.20		10.20		14.15	
	Dry wt.	Water	Dry wt.	Water	Dry wt.	Water
2	0.46	1.96	0.55	1.83	0.63	1.94
3	0.50	1.96	0.56	1.79	0.62	1.96
Ave. . . .	0.48	1.96	0.55	1.81	0.62	1.95

TABLE VI

Gossypium herbaceum. Auburn, Ala., Oct. 1, 1911. Weight of water and dry weight per 100 square centimeters of leaf at the hours indicated. Series 4, young newly developed leaves near the top of the plant; series 5, mixed, old leaves from various parts of the plant; series 6, old leaves near the top of the plant.

No.	6		10		14.30		18	
	Dry wt.	Water	Dry wt.	Water	Dry wt.	Water	Dry wt.	Water
4	0.534	1.74	0.71	1.50	0.58	1.40	0.64	1.50
5	0.50	1.66	0.54	1.36	0.54	1.24	0.56	1.38
6	0.524	1.60	0.66	1.48	0.68	1.58	0.64	1.69
	0.515	1.66	0.64	1.45	0.60	1.41	0.60	1.52

TABLE VII

Gossypium herbaceum. Auburn, Ala., Oct. 8, 1911. Weight of water and dry weight per 100 sq. cm. of leaf at the hours indicated. Series 7, material from *alternate sides* of the same leaves on ten plants; series 8, young leaves taken indiscriminately from plants in "lot 2, closely planted in rows." All leaves in both series from fresh secondary growth at the tops of plants; series 9, old leaves taken indiscriminately: marked "old."

No.	6		11.00		14.30	
	Dry wt.	Water	Dry wt.	Water	Dry wt.	Water
7	0.50	1.56	0.58	1.45	0.58	1.44
8	0.46	1.62	0.50	1.41	0.54	1.41
9	0.54	1.4	0.53	1.28	0.68	1.57
Ave. . . .	0.50	1.54	0.54	1.38	0.60	1.47

TABLE VIII

Gossypium herbaceum. Auburn, Ala., Oct. 13, 1911. Dry weight and weight of water per 100 sq. cm. of leaf at the close of 0.83 inch precipitation during the preceding 48 hours.

Series 10, material taken by a leaf punch from alternate sides of the same leaves on ten plants, not more than three circles being taken at one time from a given leaf. Young leaves (second growth) taken near the top of plant; series 11, young leaves of the same kind selected indiscriminately from "lot 2, 1911"; series 12, material taken as in (1) above from old leaves near the top of the plants.

Series	6.30		11.00		15.00	
	Dry wt.	Water	Dry wt.	Water	Dry wt.	Water
10	0.46	1.58	0.53	1.48	0.56	1.51
11	0.45	1.55	0.47	1.43	0.49	1.36
12	0.70	1.76	0.68	1.81	0.70	1.69
Ave. . . .	0.55	1.63	0.56	1.57	0.58	1.52

TABLE IX

Gossypium herbaceum. Auburn, Ala., Sept. 16–17, 1910. Average transverse dimensions of stomata of the upper and lower sides of the leaf. Extreme measurements are given in parentheses.

Hour	Average transverse diameter, micra	Hour	Average transverse diameter, micra
16	$\frac{0.7\ (4)}{0.6\ (3)}$	4	$\frac{0\ (2)}{0.5\ (4)}$
20	$\frac{0.2\ (2)}{0.2\ (3)}$	6	$\frac{0.5\ (1)}{1.0\ (1)}$
22	$\frac{0\ (3)}{0\ (3)}$	8	$\frac{1.5\ (5)}{1.2\ (4)}$
24	$\frac{0\ (1)}{0\ (1)}$	10	$\frac{1.5\ (5)}{1.5\ (4)}$
2	$\frac{0\ (3)}{0\ (2)}$	12	$\frac{1.5\ (6)}{1.2\ (4)}$

TABLE X

Gossypium herbaceum. **Auburn, Ala., Sept. 30, 1911.** Stomatal measurements **in field plants. Averages in bold face type.**

6 $\frac{\mathbf{0}}{\mathbf{0}}, \frac{0}{0}$

6.20 $\frac{0}{0}, \frac{0}{0}$

6.30 $\frac{0-2}{0-3}, \frac{0}{0}$ **(0.9)**

6.40 $\frac{0}{0-2}, \frac{0}{0-2(4)}, \frac{0}{0-2(4)}$ **(0.6)**

6.55 $\frac{\mathbf{0-4}}{\mathbf{0-4}}, \frac{0-3(4)}{0-4}$ **(2)**

7.00	$\frac{0-4(6)}{0-3(5)}$ (2)		
7.20	$\frac{0}{0-(4)}, \frac{0-3(4)}{0-4(6)}, \frac{0-(3)}{0-(4)}$ (2)		
7.25	$\frac{0-6}{0-6}, \frac{0-4}{0-6}, \frac{0-6}{0-6(7)}$ (4.5)		
7.35	$\frac{0-3(6)}{0-5(8)}, \frac{0-4}{0-6}$ (2.5)		
7.40	$\frac{0-(4)}{0-4}, \frac{0-3(6)}{(0)-5(9)}, \frac{0-4}{0-4}, \frac{0-4(6)}{0-6(8)}$ (2.5)		
7.45	$\frac{0-6(8)}{0-8}, \frac{0-4(6)}{0-4(8)}, \frac{0-8(12)}{4-12}$ (4.5)		
7.50	$\frac{0-6(8)}{0-4(6)}, \frac{0-8(10)}{0-8}, \frac{(0)-5(7)}{0-4(6)}$ (3)		
8.00	$\frac{0-5(10)}{0-3(6)}$ (2)		
8.20	$\frac{(0)4-8}{(0)4-8(10)}, \frac{0-4(8)}{0-8(12)}, \frac{(0)2-8}{0-8(12)}, \frac{0-6(8)}{0-6(10)}$ (4.5)		
8.50	$\frac{3-8}{0-8(10)}, \frac{0-6(8)}{0-6(10)}$ (4)		
8.55	$\frac{0-8}{0-10}, \frac{(0)-6(10)}{(0)-6(10)}$ (4.5)		
9.20	$\frac{0-4(8)}{0}, \frac{0-3(8)}{0}$ (2.5)	Notes.	
9.25	$\frac{0-4(8)}{0-6(8)}, \frac{0-3(4)}{0-1(4)}$ (2)		
	$\frac{0-6}{0-(4)}, \frac{0-4}{0-1(4)}$ (2)		
9.55	$\frac{0-8}{0-10}, \frac{0-1(4)}{0-1}$ (2.5)		
	$\frac{0-2}{0-1}, \frac{0-4(6)}{0-3(6)}$ (1.5)		
10.00	$\frac{0(1)}{0(1)}, \frac{0(1)}{0(1)}$ (0)	Shade	Similar leaves.
	$\frac{0-4}{0-3(6)}, \frac{0-2(4)}{0-3(4)}$ (1.5)	Sunlight	
	$\frac{0}{0}, \frac{0}{0}$ (0)	Large leaf; sunlight.	
	$\frac{0-10(12)}{0-6}, \frac{0(3)}{0-2(4)}$ (2)	Two young leaves of same age, twig, and exposure; apex of plant.	
10.35	$\frac{0-8(10)}{0-(8)}, \frac{0-1(2)}{(0)2-4(8)}$ (3)	Two mature leaves on same twig.	
	$\frac{0-1}{0}, \frac{0}{0}$ (0)	Two old leaves, apparently wilted.	
10.40	$\frac{0}{0}, \frac{0}{0}$ (0)	Two small young leaves near base of plant.	
	$\frac{0-1}{0(3)}, \frac{0-2(4)}{(0)3}$ (1.5)	Two small leaves near base of plant.	
	$\frac{(0)2-4}{0(4)}, \frac{0-1}{0}$ (0.8)		

	$\frac{0(3)}{0}, \frac{0\text{–}2(4)}{0(2)}$ (0.4)	Two very young leaves at top of plant, same light exposure.
11.00	$\frac{0(2)}{0(2)}, \frac{0(2)}{0(2\text{–}6)}$ (0)	Two mature leaves tops of neighboring plants, with same exposures.

TABLE XI

Gossypium herbaceum. Sept. 27, 1911. Transpiration during wilting. Top of chief shoot, with 3 mature leaves and 5 young leaves. Cut end vaselined. Weighed indoors in bright diffused light.

Hour	Weight, grams	Loss	Rate per minute
7.22	19.3		
27	19.06	0.24	.048
32	18.89	0.17	.034
38	18.70	0.19	.032
44	18.56	0.14	.024
50	18.46	0.10	.017
56	18.36	0.10	.017
8.02	18.26	0.10	.017
07	18.16	0.10	.020
16	18.07	0.09	.001
31	17.89	0.18	.0012
40	17.76	0.13	.0014
50	17.65	0.11	.0011
9.02	17.51	0.14	.0012
11	17.41	0.10	.0011
27	17.23	0.18	.0012

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